

Ruxolitinib for myelofibrosis

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Myelofibrosis (MF), a myeloproliferative neoplasm, is a disease associated with a significant burden of symptoms, shortened survival and an array of standard treatment regimens which have historically lacked impact and efficacy. The discovery in 2005 of the highly prevalent JAK2V617F-activating tyrosine kinase mutation, strongly associated with myeloproliferative neoplasms, led to the rapid development of a new class of drugs, JAK inhibitors, for the treatment of MF. These drugs have produced a profound effect upon splenomegaly, proliferative blood counts and constitutional symptoms, which are characteristic of MF, and have given hope to both patients and physicians who treat this debilitating disease. This article reviews the current evidence for the use of the JAK inhibitor ruxolitinib, which has completed Phase III trials and with which there is the most extensive clinical experience, as well as assessing other JAK inhibitors in clinical development.

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Myelofibrosis (MF) was first recognized as a distinct clinical entity in 1879 when Gustav Heuck published two cases of leukemia with 'peculiar blood and bone marrow' findings [1]. In the 21st century, MF is classified as a myeloproliferative neoplasm (MPN) by the WHO and is undoubtedly the most serious disease in its class [2]. It is characterized by splenomegaly, constitutional symptoms, cytopenias, progression to leukemia and significantly shortened survival. Constitutional symptoms can be extremely disabling and include pruritus, fever, night sweats and marked weight loss. A significant degree of morbidity also arises from progressive splenomegaly which causes pain, early satiety, portal hypertension and dyspnoea. Historically, we as clinicians have underestimated the disease burden of this condition and formal assessment using well established European Organization for Research and Treatment of Cancer-Quality of Life Questionnaire (EORTC-QLQ-C30) [3], as well as new tools such as the Myeloproliferative Neoplasm Symptom Assessment Form (MFN-SAF) put MF on a par with metastatic malignancy in terms of severity of symptoms [4].

MF may arise *de novo* or evolve from an antecedent MPN, namely polycythemia vera (PV) or essential thrombocythemia (ET). In this article, the term MF includes both primary MF (PMF) and post-PV or post-ET MF. Current diagnosis of PMF is based on the 2008 WHO criteria, which incorporate histopathological, clinical and molecular variables (Box 1 [2]). All three major criteria (characteristic megakaryocytic atypia, exclusion of another hematological disease, presence of a molecular marker or in its absence, no reactive cause) must be met and two minor criteria (any of leucoerythroblastosis, palpable splenomegaly, anemia and a raised lactate dehydrogenase). There are also specific criteria, which are used to confirm transformation of PV or ET to MF [5].

Survival in MF is related to the number of risk factors present as defined by the International Prognostic Scoring System (IPSS) at diagnosis or the dynamic

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Box 1. Criteria for the diagnosis of primary myelofibrosis – WHO 2008¹.**Major criteria**

- Presence of megakaryocytic proliferation and atypia^a usually accompanied by either reticulin or collagen fibrosis or in the absence of significant reticulin fibrosis, the megakaryocytic changes must be accompanied by an increased bone marrow cellularity (characterized by increased granulopoiesis and usually reduced erythropoiesis)
- Not meeting WHO criteria for PV^b, PH+ CML^c, ET, MDS^d or other myeloid neoplasms
- Demonstration of JAK2V617F or other clonal marker (e.g., MPLW515L/K) or in the absence of a clonal marker, no evidence that the bone marrow fibrosis is due to infection, autoimmune disorder or other chronic inflammatory condition^e, hairy cell leukemia or other lymphoid neoplasm, metastatic disease or toxic myelopathies^f

Minor criteria

- Palpable splenomegaly
- Anemia
- Leucoerythroblastic blood film
- Increased serum lactate dehydrogenase

¹Primary myelofibrosis requires all three major criteria and two minor criteria.

^aSmall to large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous or irregularly folded nuclei and dense clustering.

^bRequires the failure of iron replacement therapy to increase hemoglobin level to PV range in the presence of decreased ferritin. Exclusion of PV is based on hemoglobin and hematocrit levels, red cell mass is not required.

^cRequires the absence of BCR-ABL1.

^dRequires the absence of dyserythropoiesis and dysgranulopoiesis.

^ePatients with conditions associated with reactive myelofibrosis are not immune to MF and the diagnosis should be met in such cases if the criteria are met.

^fDegree of abnormality could be borderline or marked.

ET: essential thrombocythemia; MDS: myelodysplastic syndromes; MF: Myelofibrosis; PH+ CML: Philadelphia positive chronic myeloid leukemia; PV: Polycythemia vera.

IPSS/dynamic IPSS-plus during the course of the disease (Tables 1 & 2 [6–8]). The median survival from the time of diagnosis is 4 years for patients with intermediate-2 risk disease and 2 years for patients with high-risk disease [6]. Although these prognostic scores were originally validated for PMF, they are widely used for all patients with MF. Treatment options for patients with MF are varied and tend to be directed at the specific clinical need, for example, improvement of anemia (Box 2). Allogeneic stem cell transplantation remains the only curative option for patients with MF but is reserved for those with a suitable donor who are also both fit enough and willing to proceed with this risky therapy. The median of onset of MF is in the sixth decade, which renders the vast majority of patients unsuitable for transplantation. The remaining majority of patients are managed medically with treatments that have been only modestly efficacious and generally not well tolerated. Agents such as hydroxycarbamide, interferon and thalidomide have made little impact on symptoms or indeed splenomegaly [9–11] and there has been a clear need for improvement in therapeutic options. The development of a new class of agents, JAK inhibitors, offers the promise of a better quality of life for patients and the potential for improved survival, something that no other agent has yet offered.

A watershed arrived for MPNs in 2005, with the discovery of the highly prevalent JAK2V617F activating tyrosine kinase mutation [12–15]. JAK2V617F is present in over half of all MF cases but aberrant JAK2

signaling is a feature of MPNs regardless of whether JAK2V617F is mutated or not, with several other mutations including MPL (thrombopoietin receptor) and JAK exon 12 [16] also exerting their effects through JAK2 [17]. JAK2 is therefore an attractive therapeutic target and it was hoped that new therapies could be developed to treat this difficult disease in a manner akin to imatinib (Novartis; Switzerland) for chronic myeloid leukemia (CML). However, there are important caveats as discussed below in that while many patients have activation of JAK1 and JAK2 there exists a large number of underlying molecular abnormalities evident in MF, and JAK2 activation may not be the most fundamental target relevant to disease pathogenesis. In addition, unlike the *BCR/ABL* fusion gene, JAK2V617F does not generate a novel protein; instead it activates a protein whose function is crucial to normal hemopoiesis.

JAK inhibitors are, however, a perfect and gratifying example of the rapid pace at which drug development now occurs. As the JAK2V617F mutation was being described, almost simultaneously, drug development of a targeted inhibitor was initiated. Phase I trials began in mid 2007 and US FDA approval for the first JAK inhibitor was granted in late 2011. This inhibitor is ruxolitinib, which is the subject of this article.

Pathogenesis of MF

JAK2 is a member of the JAK family of cytoplasmic

tyrosine kinases, which also include JAK1, JAK3 and TYK2. They are required for signaling by cytokine and growth factor receptors that lack intrinsic kinase activity [18]. The JAK2V617F mutation is an acquired mutation involving a change from G to T in exon 14 of JAK2 that results in substitution of the normal valine residue at position 617 by more bulky phenylalanine. JAK2 is a nonreceptor tyrosine kinase (it has the ability to transfer a phosphate group from ATP to a tyrosine residue) that plays an essential role in signal transduction from several cytokine receptors that are essential for normal myelopoiesis, including the EPO receptor, the thrombopoietin receptor (MPL) and the G-CSF receptor. Wild-type JAK2 assumes an inactive conformation until binding of specific ligands to their receptors, for example, EPO to EPO receptor, which then results in a conformational change in JAK2. Activated JAK2 phosphorylates specific tyrosine kinase residues that lead to downstream signaling cascades involving STAT, MAPK and PI3K proteins. The JAK2V617F mutation results in the constitutive activation of JAK2 in the absence of cytokine receptor stimulation (i.e., in the absence of binding to cognate ligand) and uncontrolled downstream signaling. This is sufficient to produce a MPN phenotype *in vivo* when JAK2V617F is expressed in murine bone marrow cells [14]. Unlike CML, where no known vital function for endogenous ABL kinase has been identified and therefore its complete inhibition is possible, JAK2 plays a critical role in normal hemopoiesis and its inhibition would be anticipated to be associated with a degree of myelosuppression.

It is worth noting that constitutive JAK2 activation is a feature of patients with MF regardless of whether or not they have the JAK2V617F mutation, and indeed a variety of other mutations have now been described in patients with MF – as well as other MPNs and myeloid malignancies. *MPL W515L* mutations were first described in 4 out of 45 (9%) of cases of JAK2V617F mutation-negative PMF [19], an incidence confirmed by other studies [20, 21]. *MPL* mutation-positive patients were older, more frequently female and

Variable	IPSS	DIPSS	DIPSS-plus
Age >65 years	✓	✓	✓
Constitutional symptoms	✓	✓	✓
Hb <10 g/dl	✓	✓	✓
Leukocyte count >25 × 10 ⁹ /l	✓	✓	✓
Circulating blasts ≥1%	✓	✓	✓
Platelet count <100 × 10 ⁹ /l	N/A	N/A	✓
Red blood cell transfusion required	N/A	N/A	✓
Unfavorable karyotype [†]	N/A	N/A	✓
Points	1 point each	1 point each but Hb = 2 points	1 added to the DIPSS risk group* for each DIPSS-plus variable

[†]Unfavorable karyotypes: +8, -7/7q-, i(17q), inv(3), -5/5q-, 12p-, 11q23 rearrangement.
^{*}Note that this is the risk group, not the sum of points: low = 0; intermediate 1 = 1; intermediate 2 = 2; high risk = 3.
 DIPSS: Dynamic International Prognostic Scoring System; Hb: Hemoglobin; IPSS: International Prognostic Scoring System.

presented with more severe anemia [21]. Mutations in *TET2* occur in approximately 15% of cases of PMF and are associated with older age and anemia but there is no correlation with overall survival or risk of leukemia transformation [22] and *TET2* testing is not recommended on a routine basis. The clinical significance of mutations in other genes, including *IDH1/2*, *ASXL1*, *LNK*, *IKZF1*, *CBL* and *N-RAS*, remain unclear. *EZH2* mutations are seen in approximately 5% of cases and have been associated with a poor prognosis [23]. Not all of these mutations will directly activate JAK2 and they may have a variety of roles including possibly being disease-initiating or

Risk group	IPSS		DIPSS		DIPSS-plus	
	Number predictors	Median survival (years)	Number Predictors	Median survival (years)	Number predictors	Median survival (years)
Low	0	11.3	0	Not reached	0	15.4
Intermediate-1	1	7.9	1 or 2	14.2	1	6.5
Intermediate-2	2	4.0	3 or 4	4	2–3	2.9
High	>3	2.3	5 or 6	1.5	>4	1.3

DIPSS: Dynamic International Prognostic Scoring System; IPSS: International Prognostic Scoring System.

Box 2. Current treatment options for myelofibrosis.	
Clinical need	Drug/intervention
Anemia	Corticosteroids Danazol Erythropoietin Thalidomide Pomalidomide Lenalidomide
Symptomatic splenomegaly	Hydroxyurea Cladribine Splenectomy
Extramedullary hematopoiesis	Radiation therapy
Risk of thrombosis or recurrence	Low-dose aspirin Hydroxyurea
Constitutional symptoms/QOL	None specifically directed
Risk of leukemia transformation	None specifically directed
Improved survival	Allogeneic stem cell transplant (?)

QOL: Quality of live.

associated with progression.

It is clear that the pathogenesis of MF is incompletely understood and although activation of JAK2 is a common finding it is clear that the JAK2V617F mutation may not always be the disease-initiating mechanism and molecular changes occurring both before and, or indeed instead of, JAK2V617F may also be important.

Preclinical development of ruxolitinib

Ruxolitinib ([also known as INCB018424; trade name in the USA: Jakafi™; Incyte Corporation, DE, USA] US rights/Novartis AG [Basel, Switzerland]; rights outside the USA) is a potent selective and orally bio-available inhibitor of both JAK1 and JAK2 with IC₅₀ values of 3.3 and 2.8 nM, respectively [24]. Although the characteristic mutation JAK2V617F occurs within the tyrosine kinase JAK2, it is not the only kinase dysregulated in this disease. JAK1 signaling has been found to be constitutively activated in the peripheral blood of patients with MF [24]. Since many proinflammatory cytokines signal through a JAK1-dependent cellular pathway, JAK1 is also an attractive additional therapeutic target. In its preclinical development, ruxolitinib demonstrated modest selectivity against TYK2 (sixfold) and marked selectivity (>130-fold) against JAK3. No significant inhibition was observed when ruxolitinib, at a concentration of 100-fold the IC₅₀ value of JAK1 and JAK2, was tested against a commercially available panel of 26 additional kinases.

In a murine model of JAK2V617F-driven malignancy, treatment with ruxolitinib resulted in significant attenuation of spleen growth and significantly

increased mouse survival compared with mice treated with vehicle alone [24]. This was accompanied by a dramatic decrease in the cytokines, IL-6 and TNF- α . Ruxolitinib was also demonstrated to inhibit hematopoietic progenitor cells derived from CD34⁺ cells isolated from PV patients and did so more potently than with progenitor cells from normal donors [24].

Phase I & II studies

A Phase I dose-escalation study using ruxolitinib 25 mg twice a day (b.i.d.) demonstrated impressive results with regards to reduction in splenomegaly and improvement in constitutional symptoms [25]. The anticipated dose-limiting toxicity was thrombocytopenia and related to the (expected) inhibition of wild-type JAK2, which is essential for normal hemopoiesis. Nonhematological toxic effects related to therapy were infrequent (<10%) and of low grade. Pharmacodynamic and biomarker studies have shown a normalization of the exaggerated STAT3 signaling seen in MF patients in conjunction with suppression of proinflammatory cytokines such as IL-1, IL-6 and TNF- α .

This study was expanded to Phase II and enrolled 153 patients who had a median of over 14 months on study. Results demonstrated that 50% achieved an International Working Group-Myelofibrosis Research and Treatment clinical improvement response for splenomegaly [25]. Responses for hepatomegaly were more modest. White-cell counts were reduced from $29.8 \times 10^9/l$ at baseline to $16.0 \times 10^9/l$ after 3 months ($p = 0.001$), and remained stable through 1 year. Sixteen of 17 patients with elevated platelet counts at baseline (mean: $728 \times 10^9/l$) had reduced platelet counts at 3 months (mean: $336 \times 10^9/l$). Median weight gain after 1 year in patients receiving ruxolitinib 15 and 20 mg b.i.d. were 9.4 and 7.1 kg, respectively. Furthermore, weight gain was more prominent in those with BMI in the lowest quartile at baseline versus those in the highest quartile. Performance status was gradually improved and was generally maintained. Standardized 6 min walk tests were performed on 27 patients after one, three and six cycles of treatment, and the mean distances walked were 34, 57 and 71 m. In addition, serial collections of the MF-Symptom Assessment Form throughout this trial demonstrated significant improvement in MF-associated symptoms. The greatest improvements occurred with regard to abdominal discomfort, night sweats, pruritus and fever. A reduction in signal transduction and proinflammatory cytokine levels, presumably through JAK1 and JAK2 inhibition, paralleled improvements in the patients' symptoms (a reduction in composite symptom score after therapy). Results were equivalent irrespective of JAK2 mutational status or subtype of MF. There was only a

modest decrease in mutant JAK2 allele burden despite significant clinical benefit, suggesting that the mode of action may be through inhibition of JAK1 signaling and subsequent reduction in inflammatory cytokines, as well as aberrant JAK2 and not due to a frank decrease in allele burden [25]. In a move that has now determined strategy for future clinical trials in this field, the investigators evaluated spleen volume by MRI, equating a median reduction in MRI-measured spleen volume of 33% and median reduction in spleen length of 52% [25].

Phase III studies: the COMFORT trials

Ruxolitinib has been evaluated in Phase III trials known as the COMFORT trials. COMFORT-I was a randomized, double-blind study evaluating the efficacy and safety of ruxolitinib in patients with PMF and post-PV/ET-MF, whereas COMFORT-II was a randomized, open-label study comparing the efficacy, safety and tolerability of ruxolitinib versus best available therapy (BAT) in patients with PMF and post-PV/ET-MF. These trials were reported at the American Society of Clinical Oncology and European Hematology Association meetings in 2011, and have recently been published in the *New England Journal of Medicine* [26, 27].

COMFORT-I included 309 adult MF patients randomized 1:1 to ruxolitinib or placebo. Patients in the ruxolitinib arm received 15 mg b.i.d. (patients with platelet count $100\text{--}200 \times 10^9/l$) or 20 mg b.i.d. (patients with platelet count $>200 \times 10^9/l$). The proportion of patients with spleen volume reduction $\geq 35\%$ evaluated by MRI or computed tomography (CT) at week 24 (primary end point) was 41.9% with ruxolitinib versus 0.7% with placebo ($p < 0.0001$) [27]. Indeed, the sole responding placebo patient had sustained a splenic infarct – which accounted for the reduced spleen volume – and died shortly thereafter. At week 24, as measured by the modified Myelofibrosis Symptom Assessment Form v2.0 [28], 45.9% of patients receiving ruxolitinib versus 5.3% of those receiving placebo ($p < 0.0001$) experienced symptom alleviation by at least 50% reduction in their total symptom score. Mean total symptom score improved by 46.1% in the ruxolitinib arm, compared with a worsening of 41.8% in this score in the placebo arm ($p < 0.0001$). In contrast with the worsening of all individual symptoms observed in the placebo arm, each symptom that comprised the total symptom score (abdominal discomfort, pain under left ribs, early satiety, night sweats, itching, musculoskeletal pain and inactivity) improved significantly with ruxolitinib treatment. Quality of life (QOL), measured by EORTC-QLQC30 improved with symptom alleviation [29]. Ten ruxolitinib patients and 14 placebo patients died. The study is ongoing and neither the median duration of response nor the median

survival on the active (ruxolitinib) arm have yet been reached.

COMFORT-II included 219 adult MF patients from nine European countries, randomized 2:1 to ruxolitinib or BAT. Patients in the ruxolitinib arm received a starting dose of ruxolitinib 15 or 20 mg b.i.d. (as per the COMFORT-I trial), with a possibility of dose titration ranging from 5 to 25 mg b.i.d. The proportion of patients with spleen volume reduction $\geq 35\%$ evaluated by MRI or CT at week 48 (primary end point) was 28.5% with ruxolitinib versus 0% with BAT ($p < 0.0001$) [26]. The proportion of patients with spleen volume reduction $\geq 35\%$ evaluated by MRI or CT at week 24 (key secondary end point and equivalent to the primary end point of COMFORT-I) was 31.9% with ruxolitinib versus 0% with BAT ($P < 0.0001$). The median duration of response was not reached. Mean improvements from baseline in Functional Assessment of Cancer Therapy–Lymphoma System [30] subscores were greater in the ruxolitinib arm, indicating better QOL versus patients receiving BAT. The EORTC-QLQ-C30 scores for symptoms relevant to MF patients showed improvement from baseline by week 8 and continued through week 48, also indicating improvement in QOL. Ten and four deaths occurred in the ruxolitinib and BAT arms, respectively. The study is ongoing and the results of progression-free survival, leukemia-free survival, overall survival and change in bone marrow histomorphology are not significantly different between the two arms as yet.

In the 2011 American Society of Hematology meeting updates from these trials, suggested that patients benefitted across all subgroups (PMF, post-ET MF, PV MF) regardless of JAK2V617F mutation status, gender or IPSS score [31,32]. Importantly, the COMFORT-I trial now shows a clear survival advantage with 13 ruxolitinib- and 24 placebo-treated patients dying during the study or during extended follow-up (median follow up of 52 and 51 weeks, respectively), representing a hazard ratio (95% CI) of 0.499 (0.254 and 0.98; $p = 0.0395$). For ruxolitinib- and placebo-treated patients, respectively, the probability of survival (95% CI) beyond 48 weeks was 0.98 (0.92 and 0.99) and 0.90 (0.81 and 0.95) for patients with baseline hemoglobin values ≥ 10 g/dl and 0.84 (0.72 and 0.91) and 0.77 (0.63 and 0.86) for patients with baseline hemoglobin < 10 g/d [31]. This was also shown in a comparison between the MD Anderson Cancer Center (TC, USA) cohort of the Phase I/II ruxolitinib-treated patients and a matched historical cohort with clinical characteristics that would have allowed them to participate in the Phase I/II study of ruxolitinib [33]. The survival of patients with high-risk MF that were treated with ruxolitinib was found to be

Table 3. JAK inhibitors currently under development.

Compound	IC ₅₀ (nm) for JAK family				Current status of development
	JAK1	JAK2	JAK3	TYK2	
Lestaurtinib	3	1	NA	NA	Phase II
Ruxolitinib	2.8	4.5	322	30	Phase III Approved by the US FDA and European Medicines Agency
SAR302503/ TG101348/	105	3 (19 vs JAK2V617F)	>1000	405	Phase III
XL019	134	2	195	344	Halted
Pacritinib (SB1518) [†]	1.7	1.8	0.75	260	Phase II/III [†]
CYT387	11	18	155	17	Phase I/II
LY2784544	NA	NA	NA	NA	Entering Phase II
INCB028050	5.9	5.7	560	53	Phase II [†]
INCB016562	2.5	0.3	10.8	2.7	Preclinical
NVP-BSK805	31.6	0.48	18.6	10.7	Preclinical
R723	>1000	2	24	NA	Preclinical

[†]Values expressed as K_d (dissociation constant).

[†]Tested only in rheumatologic diseases, not in myeloproliferative neoplasm.

significantly longer than that of the matched control group ($p = 0.022$). Furthermore, ruxolitinib therapy was identified as an independent factor influencing better survival in the multivariate analysis. These data suggest the potential of ruxolitinib to change the natural progression of MF even in patients with advanced disease and argue strongly for the inclusion of this agent into the therapeutic options.

Toxicity & safety

Concerning toxicity and safety of ruxolitinib, in the Phase I/II clinical trial thrombocytopenia was reported as the dose-limiting toxicity, which was dose-dependent and reversible [25]. The likely mechanism of action of thrombocytopenia post-ruxolitinib is via JAK2 inhibition affecting thrombopoietin signaling, though this has not been fully elucidated. In addition, of patients who were transfusion-independent at baseline, new-onset anemia occurred in 23% and was dose-dependent [25]. Nonhematologic toxic effects were infrequent and occurred in less than 10% of patients (e.g., asthenia [2.0%], with fatigue, anxiety, fever and insomnia [each 1.3%]). Two patients (1.3%) with a history of cardiopulmonary disease developed a clinical picture assessed by an investigator as 'systemic inflammatory response syndrome (SIRS)-like event' after abrupt cessation of ruxolitinib. Mayo Clinic (MN, USA) investigators recently published five cases developing a SIRS-like clinical picture following drug withdrawal and a high rate of drug discontinuations in this clinical center; however, some of these patients were on higher doses of drug than now used in standard

practice [34]. Importantly, a SIRS-like constellation of events has not been observed in the larger Phase III COMFORT clinical trials [26, 27], where there were substantially greater numbers of patients observed for prolonged periods treated at overall lower ruxolitinib doses. The most commonly reported nonhematologic adverse effects of all grades irrespective of causality in COMFORT-I (ruxolitinib vs placebo) were fatigue (25 vs 34%), diarrhoea (23 vs 21%), ecchymosis (19 vs 9%), dizziness (15 vs 7%) and headache (15 vs 5%), and in COMFORT-II (ruxolitinib vs BAT), diarrhoea (23 vs 11%) and peripheral edema (22 vs 26%). In these pivotal trials, discontinuations due to adverse effects were 11 vs 11% (ruxolitinib vs placebo) and 8.2 vs 5.5% (ruxolitinib vs BAT), attesting to the high degree of safety and tolerability of ruxolitinib compared with current agents.

Current situation

Ruxolitinib was approved by the FDA for treatment of intermediate- or high-grade MF on 16 November 2011 and just recently by the European Medicines Agency. It is currently being considered by health authorities in other areas of the world. There are several ongoing trials and the drug is available from Novartis for compassionate use outside the USA.

Use of ruxolitinib in conditions other than MF

Potential other uses for ruxolitinib may include relapsed/refractory acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, CML, or blastic-phase or tyrosine-kinase-refractory

CML, select subtypes of lymphomas, relapsed or refractory assorted solid tumors, and inflammatory conditions such as rheumatoid arthritis and Castelman's disease. Studies evaluating these agents in some of these conditions are ongoing. Future indications for ruxolitinib also include other MPNs, in particular PV. Standard therapies for high-risk patients with ET and PV, although generally well tolerated, can be problematic, and there is a need to develop agents for use when resistance or intolerance to these standard agents occurs [35]. Ruxolitinib has already been evaluated in 39 ET and 34 PV patients. Verstovsek *et al.* reported similar rates of reduction of splenomegaly and symptom scores but in this case all patients had leucocyte counts below $10 \times 10^9/l$ and 41% achieved a complete response (per standard response criteria by the European LeukaemiaNet) with platelets $<400 \times 10^9/l$; no thrombotic events have been reported in PV patients in this Phase II trial [36]. Specifically with regard to the ET subcohort, 49% of ET patients achieved normal platelet counts and 79% achieved $<600 \times 10^9/l$ or a $\geq 50\%$ reduction as of their

last follow-up visit, while 13 of 14 subjects with baseline platelet counts $>1000 \times 10^9/l$ achieved a greater than 50% reduction. Moreover, this study demonstrated marked and sustained benefit of this agent upon symptoms of grave concern in PV/ET patients, in particular pruritus but also fatigue. In PV patients refractory or intolerant of hydroxycarbamide a trial termed 'RESPONSE' is currently underway to assess the utility of ruxolitinib compared with BAT [101].

Conclusion

Ruxolitinib was evaluated within 2 years of the first description of the JAK2V617F mutation and approved by the FDA within less than 6 years. The evidence presented here suggests that this drug has a profound effect upon splenomegaly, proliferative blood counts and constitutional symptoms. There is also emerging evidence that while cure is not achieved, patients treated with ruxolitinib survive longer. Ruxolitinib thus represents a highly significant therapeutic advance that has the potential to benefit a large number of patients with MF and possibly other MPNs.

Executive summary

Myelofibrosis pathogenesis & characteristics

- Myelofibrosis (MF) is a rare blood cancer.
- Characteristic features are splenomegaly, debilitating symptoms (including constitutional symptoms) and shortened survival.
- The pathogenesis of MF is not completely understood; JAK2V617F is a JAK2-activating mutation found in over 50% of patients.
- Activation of the JAK-STAT pathway is a nearly universal finding in MF, but JAK2V617F may not always be the disease-initiating mechanism.
- Other molecular changes occurring both before and/or indeed instead of JAK2V617F may also be important.

Ruxolitinib: Phase I & II studies

- Ruxolitinib is a potent oral JAK1 and JAK2 inhibitor.
- Phase I/II studies demonstrated marked reduction in splenomegaly and improvement in constitutional symptoms, which occurred regardless of the patients' JAK2V617F mutation status.
- Dose-limiting toxicity was thrombocytopenia.
- Nonhematological toxicity was infrequent and not severe.

Phase III studies: the COMFORT trials

- Phase III studies have recently been completed and they were known as the COMFORT trials.
- COMFORT-I was a placebo-controlled, randomized (1:1), double-blind clinical study.
- COMFORT-II was randomized (2:1), open-label clinical study comparing ruxolitinib with standard therapies.
- Both studies confirmed results from Phase I/II studies with durable statistically and clinically significant results compared with placebo and standard therapies.
- A planned analysis of COMFORT-I demonstrated a survival advantage with a median ruxolitinib treatment duration (and follow-up) of 44 weeks.

Safety & toxicity

- Anemia and thrombocytopenia are the commonest dose-limiting effects.
- These were rare causes for drug discontinuation in Phase III trials.
- A systemic inflammatory response syndrome-like clinical picture has been described upon abrupt drug withdrawal by a single center, but acute severe adverse events were distinctly uncommon in the Phase III trials (where no such systemic inflammatory response syndrome-like effect was seen upon drug discontinuation or hold). More commonly, MF-related symptoms returned to baseline within approximately 7 days after stopping or withholding ruxolitinib.

Future perspective

Ruxolitinib has only been tested to date in MF patients with IPSS intermediate risk-2 or above and there is both rationale and need to test this agent in patients with earlier stage disease. In addition, since ruxolitinib is so well tolerated, it readily lends itself to being tested in combination with other agents, which might address either, for example, the issue of ruxolitinib-induced anemia or other disease-related targets not influenced by JAK inhibition, such as the epigenome. Such studies in MF are currently underway, as are trials in MF patients with platelet counts below those tested in the ruxolitinib Phase III trials.

There are other JAK inhibitors that have different on- and off-target effects, which are at varying stages of development. These are listed in Table 3. It remains to be seen whether any of these agents will be

superior to ruxolitinib, but some show early promise; for example, CYT387 appears to improve anemia, as assessed per International Working Group response criteria. For the first 60 patients completing at least three cycles of CY387 treatment, responses were reported as follows: 45% spleen size reduction; 50% 'anemia response' and >50% constitutional symptoms response. A signal was again seen in that 58% of transfusion-dependent patients became transfusion-independent for >12 weeks. This patient group importantly included patients previously treated with ruxolitinib, SAR302503 and pomalidomide [37]. SAR302503 may reduce JAK2V617F allele burden and may in some patients reduce bone marrow fibrosis. After 24 cycles of treatment, the median allele burden was 21% (range 6–100%) compared with 60% (23–100%) at baseline ($p = 0.03$) [38]. These

data are of significant clinical interest but need to be robustly demonstrated in Phase III multicenter randomized controlled trials.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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